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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : A61K 38/13, 31/445, 31/71 // (A61K 38/13, 31:71) (A61K 38/13, 31:445) (A61K 31/71, 31:71) (A61K 31/71, 31:445) (A61K 31/445, 31:445)</p>	<p>A1</p>	<p>(11) International Publication Number: WO 98/04279 (43) International Publication Date: 5 February 1998 (05.02.98)</p>																		
<p>(21) International Application Number: PCT/EP97/04123 (22) International Filing Date: 29 July 1997 (29.07.97) (30) Priority Data: 9615942.1 30 July 1996 (30.07.96) GB 9705684.0 18 March 1997 (18.03.97) GB (71) Applicant (for all designated States except US): NOVARTIS AG (CH/CH); Schwarzwaldallee 215, CH-4058 Basel (CH). (72) Inventors; and (75) Inventors/Applicants (for US only): ZENKE, Gerhard [DE/DE]; Adolf-Glattacker-Strasse 8, D-79618 Rheinfelden (DE). SCHUURMAN, Hendrik, J. [NL/CH]; Breisacherstrasse 22, CH-4057 Basel (CH). HAEGERLIN, Barbara [CH/CH]; Haselrain 77, CH-4125 Riehen (CH). MEINZER, Armin [DE/DE]; Fliederweg 2, D-79426 Buggingen (DE). (74) Agent: ROTH, Bernhard, M.; Novartis AG, Patent- und Markenabteilung, Klybeckstrasse 141, CH-4002 Basel (CH).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>																			
<p>(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF TRANSPLANT REJECTION, AUTOIMMUNE OR INFLAMMATORY CONDITIONS COMPRISING CYCLOSPORIN A AND 40-O-(2-HYDROXYETHYL)-RAPAMYCIN</p>																				
<p>(57) Abstract</p> <p>A synergistic combination of an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506) and 40-O-(2-hydroxyethyl)-rapamycin is provided, which is useful in the treatment and prevention of transplant rejection and also certain autoimmune and inflammatory diseases, together with novel pharmaceutical compositions comprising an IL-2 transcription inhibitor in combination with a rapamycin, e.g., 40-O-(2-hydroxyethyl)-rapamycin.</p> <div data-bbox="808 1161 1385 1738"> <table border="1"> <caption>Data points estimated from the graph</caption> <thead> <tr> <th>40-O-(2-Hydroxyethyl)-rapamycin (IC₅₀ units)</th> <th>Cyclosporin A (IC₅₀ units)</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>1.0</td> </tr> <tr> <td>0.2</td> <td>0.55</td> </tr> <tr> <td>0.3</td> <td>0.45</td> </tr> <tr> <td>0.4</td> <td>0.38</td> </tr> <tr> <td>0.55</td> <td>0.25</td> </tr> <tr> <td>0.75</td> <td>0.15</td> </tr> <tr> <td>0.85</td> <td>0.12</td> </tr> <tr> <td>1.0</td> <td>0.0</td> </tr> </tbody> </table> </div>			40-O-(2-Hydroxyethyl)-rapamycin (IC ₅₀ units)	Cyclosporin A (IC ₅₀ units)	0.0	1.0	0.2	0.55	0.3	0.45	0.4	0.38	0.55	0.25	0.75	0.15	0.85	0.12	1.0	0.0
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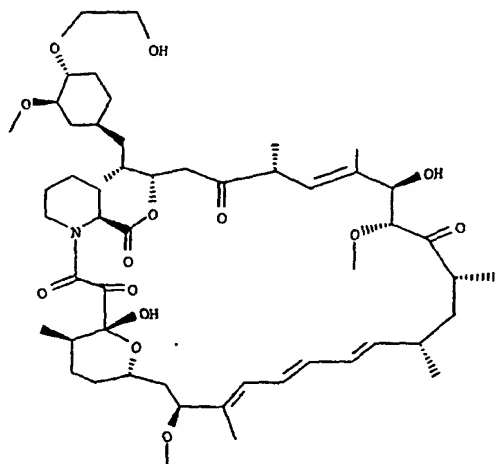
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PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF TRANSPLANT REJECTION,
AUTOIMMUNE OR INFLAMMATORY CONDITIONS COMPRISING CYCLOSPORIN A AND 40-O-
(2-HYDROXYETHYL)-RAPAMYCIN

This invention relates to certain novel pharmaceutical compositions comprising a rapamycin, e.g., 40-O-(2-hydroxyethyl)-rapamycin, and an IL-2 transcription inhibitor, in particular FK-506 or cyclosporin A, and to synergistic combinations of an IL-2 transcription inhibitor and 40-O-(2-hydroxyethyl)-rapamycin.

40-O-(2-hydroxyethyl)-rapamycin has the following structure:



Cyclosporin A (also known as Ciclosporin or Cyclosporine) is an immunosuppressive, cyclic undecapeptide. Its structure is disclosed, e.g., in The Merck Index, 11th Edition; Merck & Co., Inc.; Rahway, New Jersey, USA (1989) under listing 2759. Formulations of cyclosporin A are available commercially under the trademark SANDIMMUN or SANDIMMUNE, and a microemulsion preconcentrate formulation of cyclosporin A is sold under the trademark NEORAL or OPTORAL. Cyclosporin A is widely used as an immunosuppressant, e.g., in the prevention and treatment of graft rejection following organ transplant and of graft versus host disease, e.g., following bone marrow transplant. At higher dosages, however, it may affect kidney and liver function. Moreover, cyclosporin A is difficult to formulate, as it is essentially insoluble in most pharmaceutically acceptable solvents, e.g. aqueous pharmaceutical systems, and its oral bioavailability in most formulations is variable. Finally, although cyclosporin A is highly effective in preventing and treating acute rejection episodes in transplant patients and hence contributes to long-term graft survival, chronic rejection, manifest as arteriosclerosis due to vascular smooth muscle proliferation in the graft (graft-vessel disease), remains a serious problem for some patients after transplantation, for example heart transplant recipients. Cyclosporin A, which inhibits primarily T-cells, is also not particularly effective to prevent antibody-mediated rejection as is seen following xenotransplantation.

FK506 is a macrolide immunosuppressant producible by Streptomyces tsukubaensis No 9993. The structure of FK506 is given in the appendix to the Merck Index, supra, as item A5. FK506 is also used as an immunosuppressant. Although it is structurally very different from cyclosporin A, it has a similar mechanism of action, i.e., inhibition of T-cells via cytokine suppression, in particular IL-2 suppression. It is somewhat more potent than cyclosporin A, but also more toxic, and is also difficult to formulate, having low solubility and variable bioavailability and metabolism.

Immunosuppressive compounds whose immunosuppressive activity derives principally or in significant part from their direct or indirect inhibition of IL-2 gene transcription (e.g., corticosteroids, ascomycins, and cyclosporins; in particular cyclosporin A, FK506, and their various immunosuppressive derivatives and analogues; especially compounds which are at least as active as cyclosporin A in an IL-2 reporter gene assay) are hereinafter referred to as "IL-2 transcription inhibitors".

It is now surprisingly discovered that IL-2 transcription inhibitors and 40-O-(2-

hydroxyethyl)-rapamycin, in particular cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin, act synergistically, so that effective immunosuppression is seen upon co-administration at dosages which would be well below the effective dosages individually. Moreover, this synergistic combination is useful to treat, e.g. ameliorate, or prevent not only acute rejection, but also chronic rejection and xenograft rejection. Co-administration of the two compounds in synergistically effective amounts allows for significantly lower dosages of each compound in immunosuppression, thereby reducing the side effects, and by preventing chronic rejection and xenograft rejection, enhances the pharmaceutical utility of the treatment.

Synergy is calculated as described in Berenbaum, Clin. Exp. Immunol. (1977) 28: 1, using an interaction term to correct for differences in mechanism between the two drugs, as described in Chou, et al. Transpl. Proc. (1994) 26: 3043. The index of synergy is calculated as

$$\frac{\text{Dose of A}}{A_E} + \frac{\text{Dose of B}}{B_E} + \frac{(\text{Dose A}) \times (\text{Dose B})}{A_E \times B_E}$$

in which the doses of the compounds A and B represent those used in a particular combination, and A_E and B_E are the individual doses of A and B respectively giving the same effect. If the result is less than 1, there is synergy; if the result is 1, the effect is additive; if the result is greater than 1, A and B are antagonistic. As described below, cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin show an index of synergy of from about 0.3 to about 0.7 in vivo, and about 0.8 in vitro. By plotting an isobologram of dose of A / A_E vs. dose of B / B_E , the combination of maximum synergy can be determined. The synergistic ratio expressed in terms of the ratio by weight of the two compositions at synergistic amounts along this isobologram, especially at or near the point of maximum synergy, can then be used to determine formulations containing an optimally synergistic ratio of the two compounds.

Remarkably, IL-2 transcription inhibitors and 40-O-(2-hydroxyethyl)-rapamycin exhibit synergy at two levels. At a mechanistic level e.g., as seen in in-vitro results, the intrinsic immunosuppressive activity of the two compounds is synergistically enhanced on co-administration. Moreover, at a pharmacokinetic level, the observed blood levels of both compounds on co-administration are significantly improved over blood levels achieved by administration of either compound individually and correspondingly, the observed in vivo synergy is greater even than would be predicted based on the in vitro results. The mechanistic synergy in combination with the pharmacokinetic interaction synergy is extremely surprising, and indeed, this

combination of drugs is believed to be the first reported wherein significant synergy exists at both the mechanistic level and the pharmacokinetic level. The practical effect of this from the patient's perspective is that both drugs are more effective, at lower dosages, with fewer side effects, and improved bioavailability. Surprisingly, it is feasible that the drugs can be formulated into a fixed combination, which greatly enhances the convenience for the patient.

The indications for which this combination is of interest include in particular autoimmune and inflammatory conditions and conditions associated with or causal to transplant rejection, e.g., treatment (including amelioration, reduction, elimination or cure of etiology or symptoms) or prevention (including substantial or complete restriction, prophylaxis or avoidance) of the following:

- a) Acute organ or tissue transplant rejection, e.g. treatment of recipients of e.g. heart, lung, combined heart-lung, liver, kidney, pancreatic, skin, bowel, or corneal transplants, especially prevention and/or treatment of T-cell mediated rejection, as well as graft-versus-host disease, such as following bone marrow transplantation.
- b) Chronic rejection of a transplanted organ, in particular, prevention of graft vessel disease, e.g., characterized by stenosis of the arteries of the graft as a result of intima thickening due to smooth muscle cell proliferation and associated effects.
- c) Xenograft rejection, including the acute, hyperacute or chronic rejection of an organ occurring when the organ donor is of a different species from the recipient, most especially rejection mediated by B-cells or antibody-mediated rejection.
- d) Autoimmune disease and inflammatory conditions, in particular inflammatory conditions with an etiology including an immunological or autoimmune component such as arthritis (for example rheumatoid arthritis, arthritis chronica progrediente and arthritis deformans) and other rheumatic diseases. Specific autoimmune diseases for which the synergistic combination of the invention may be employed include, autoimmune hematological disorders (including e.g. hemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, polychondritis, sclerodoma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, (autoimmune) inflammatory bowel disease (including e.g. ulcerative colitis and Crohn's disease), endocrine ophthalmopathy, Graves disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis

sicca and vernal keratoconjunctivitis, interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis (with and without nephrotic syndrome, e.g. including idiopathic nephrotic syndrome or minimal change nephropathy) and juvenile dermatomyositis. Autoimmune and inflammatory conditions of the skin are also considered to be amenable to treatment and prevention using the synergistic combination of the invention, e.g., psoriasis, contact dermatitis, atopic dermatitis, alopecia areata, erythema multiforma, dermatitis herpetiformis, scleroderma, vitiligo, hypersensitivity angiitis, urticaria, bullous pemphigoid, lupus erythematosus, pemphigus, epidermolysis bullosa acquisita, and other inflammatory or allergic conditions of the skin, as are inflammatory conditions of the lungs and airways including asthma, allergies, and pneumoconiosis.

The invention thus provides products and methods for co-administration of an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506) and 40-O-(2-hydroxyethyl)-rapamycin, especially cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin, at synergistically effective dosages, e.g.,

1. A method of treatment or prevention of a condition as described above, e.g., an autoimmune or inflammatory condition or transplant rejection, especially chronic rejection or xenograft rejection, in a subject suffering from or at risk for such condition or rejection, for example a patient suffering from an autoimmune or inflammatory condition or a transplant recipient, comprising co-administering synergistically effective amounts of cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin.
2. The use of an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A) in the manufacture of a medicament for co-administration in synergistically effective amounts with 40-O-(2-hydroxyethyl)-rapamycin, e.g., for use in the treatment or prevention of a condition as described above, e.g., an autoimmune or inflammatory condition, or transplant rejection, especially chronic rejection or xenograft rejection.
3. The use of 40-O-(2-hydroxyethyl)-rapamycin in the manufacture of a medicament for co-administration in synergistically effective amounts with an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A), e.g., for use in the treatment or prevention of a condition as described above, e.g., an autoimmune or inflammatory condition, or transplant rejection, especially chronic rejection or xenograft rejection.
4. A kit of parts comprising an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506,

especially cyclosporin A) and 40-O-(2-hydroxyethyl)-rapamycin in separate unit dosage forms, preferably wherein said unit dosage forms are suitable for administration of the two compounds in synergistically effective amounts, together with instructions for use, e.g., in treatment or prevention of a condition as described above, e.g., an autoimmune or inflammatory condition, or transplant rejection, especially chronic rejection or xenograft rejection. The kit may further comprise means for facilitating compliance with the administration of the compounds, e.g. a label or drawings.

5. The use of an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A) in the manufacture of a pharmaceutical kit which is to be used for facilitating co-administration with 40-O-(2-hydroxyethyl)-rapamycin.

6. The use of 40-O-(2-hydroxyethyl)-rapamycin in the manufacture of a pharmaceutical kit which is to be used for facilitating co-administration with an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A).

7. An IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A) and 40-O-(2-hydroxyethyl)-rapamycin as a combined pharmaceutical preparation for simultaneous, separate or sequential use, preferably in synergistically effective amounts, e.g., for the treatment or prevention of a condition as described above, e.g., an autoimmune or inflammatory condition, or transplant rejection, especially chronic rejection or xenograft rejection.

8. A pharmaceutical composition comprising an IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A) and 40-O-(2-hydroxyethyl)-rapamycin, e.g., in synergistically effective amounts, in combination or association with a pharmaceutically acceptable diluent or carrier, e.g. for use in treatment or prevention of a condition as described above, e.g., the treatment or prevention of an autoimmune or inflammatory condition, or transplant rejection, especially chronic rejection or xenograft rejection.

By "synergistically effective amounts" is meant an amount of IL-2 transcription inhibitor and an amount of 40-O-(2-hydroxyethyl)-rapamycin which are individually below their respective effective dosages for the relevant indication, but which are pharmaceutically active on co-administration, e.g., in a synergistic ratio, for example as calculated above. Furthermore, "synergistically effective amounts" may mean an amount of IL-2 transcription inhibitor and an amount of 40-O-(2-hydroxyethyl)-rapamycin which are individually equal to their respective effective dosages for the relevant indication, and which result in a more than additive effect. The molar amount of 40-O-(2-hydroxyethyl)-rapamycin present is significantly less, preferably one half

or less, than the amount of IL-2 transcription inhibitor. Synergistic ratios of cyclosporin A to 40-O-(2-hydroxyethyl)-rapamycin by weight are thus suitably from 2:1 to 180:1, preferably from 5:1 to 50:1, most preferably from 10:1 to 20:1, e.g. about 16:1. Synergistic ratios of cyclosporin A to 40-O-(2-hydroxyethyl)-rapamycin by weight are for example 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 21:1, 22:1, 23:1, 24:1, 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 36:1, 37:1, 38:1, 39:1, 40:1, 41:1, 42:1, 43:1, 44:1, 45:1, 46:1, 47:1, 48:1, 49:1, 50:1 or greater than 50:1, e.g. 60:1.

Absolute dosages of the compounds will vary depending on the individual, the route of administration and the nature and severity of the condition to be treated. For example, in prevention and treatment of transplant rejection, an initial dosage of about 2-3 times the maintenance dosage is suitably administered 4-12 hours prior to transplantation, followed by a daily dosage of about 2-3 times the maintenance dosage for a period of from 1-2 weeks, and subsequently the dose is gradually tapered down at a rate of about 5% per week to reach the maintenance dosage. In general, synergistically effective amounts of 40-O-(2-hydroxyethyl)-rapamycin and cyclosporin A on oral administration for use in prevention and treatment of transplant rejection in larger animals, e.g., man, are amounts of cyclosporin A of up to 5 mg/kg/day, e.g., from 0.5 mg/kg/day to 3 mg/kg/day, preferably about 1.5 mg/kg/day, in combination or co-administration with amounts of 40-O-(2-hydroxyethyl)-rapamycin of up to 2.5 mg/kg/day, e.g., from 0.01 mg/kg/day to 1 mg/kg/day, preferably about 0.1 mg/kg/day, in a synergistic ratio, as described. Suitable unit dosage forms for oral co-administration of these compounds thus may contain on the order of from 0.1 to 70 mg, preferably 1.0 to 10.0 mg, of 40-O-(2-hydroxyethyl)-rapamycin and from 1 to 200 mg, preferably 10 to 100 mg of cyclosporin A. The daily dosage for oral administration is preferably taken in a single dose, but may be spread out over two, three or four dosages per day. For i.v. administration, the effective dosage is lower than that required for oral administration, e.g. about one third the oral dosage. Because the bioavailability of cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin is subject to a certain degree of individual variability, it may be advisable to measure blood levels of the cyclosporin and/or 40-O-(2-hydroxyethyl)-rapamycin, preferably using monoclonal antibody-based assays as are known in the art for both cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin, especially during the first few months, in order to establish an optimal maintenance dosage for the individual patient.

By "co-administration" is meant administration of the 40-O-(2-hydroxyethyl)-rapamycin

and the IL-2 transcription inhibitor, e.g., cyclosporin A, together or at substantially the same time (e.g., within fifteen minutes or less), either in the same vehicle or in separate vehicles, so that upon oral administration, for example, both compounds are present simultaneously in the stomach. Preferably, the compounds are administered as a fixed combination, e.g., a pharmaceutical formulation according to 5 above.

Pharmaceutical compositions under 6 above include compositions suitable for administration by any conventional route, in particular compositions suitable for administration enterally, e.g. orally, for example in the form of solutions for drinking, tablets or capsules, or parenterally, for example in the form of injectable solutions or suspensions. Normally for systemic administration oral dosage forms are preferred, although for some conditions, for example for prevention of rejection of liver transplants, an intravenously injectable form is desirable. Pharmaceutical compositions under 6 above also include compositions suitable for topical administration e.g., in the form of a dermal cream, ointment, gel or like preparation, especially in combination or association with penetration enhancing agents, e.g., for the treatment of autoimmune or inflammatory conditions of the skin, as well as composition in the form of an ocular cream, gel or eye-drop preparation, e.g. for the purposes of application to the eye, and inhalable compositions, e.g., for use in treatment of autoimmune or inflammatory conditions of the lungs and airways.

Pharmaceutical compositions under 6 above, e.g., for oral administration, are suitably emulsions, microemulsions, emulsion preconcentrates or microemulsion preconcentrates, or solid dispersions, especially water-in-oil microemulsion preconcentrates or oil-in-water microemulsions, comprising the IL-2 transcription inhibitor (e.g., cyclosporin A or FK506, especially cyclosporin A) and 40-O-(2-hydroxyethyl)-rapamycin in a synergistic ratio.

An emulsion preconcentrate is a formulation which forms an emulsion in an aqueous medium, e.g., water or gastric juice. An emulsion is an opaque or substantially opaque colloidal dispersion that is formed when its components are brought into contact, e.g., a composition containing dispersed particles of a size greater than about 2000 Å (200 nm) in diameter, e.g. as described in GB 2 270 842, the contents of which are incorporated herein by reference. A microemulsion preconcentrate is a formulation which spontaneously forms a microemulsion in an aqueous medium, e.g., water or gastric juice. A microemulsion is a transparent or slightly opalescent colloidal dispersion that is formed spontaneously or substantially spontaneously when its

components are brought into contact, e.g., a thermodynamically stable composition containing dispersed droplets of a size less than about 2000 Å (200nm) in diameter, generally less than 1500 Å (150 nm), typically from 30 to 1000 Å (3 to 100nm), for example as described in GB 2 222 770 A.

Preferred compositions are those having cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin in a synergistically effective ratio in an oil-in-water microemulsion or in a water-in-oil microemulsion preconcentrate capable of forming a microemulsion, comprising a hydrophilic phase, a lipophilic phase, and a surfactant, e.g. wherein the hydrophilic phase, lipophilic phase, and surfactant are as described in GB 2 222 770, GB 2 257 359 or in WO 96/13273 the contents of which publications are incorporated herein by reference. The hydrophilic phase may comprise 5 to 50 % by weight of the composition, e.g. 10 to 50%; preferably 15 to 40 % by weight. The lipophilic phase may comprise 5 to 85 % by weight of the composition, e.g. 10 to 85%; preferably 15 to 70 % by weight. The surfactant may comprise 5 to 80 % by weight of the composition; preferably 10 to 70% by weight.

Suitable components for the hydrophilic phase include components described in GB 2 222 770, for example a pharmaceutically acceptable C₁₋₅ alkyl or tetrahydrofurfuryl di- or partial-ether or a low molecular weight mono-or poly-oxy-alkanediol, e.g. diethylene glycol monoethyl ether available commercially under the trade name Transcutol, or tetrahydrofurfuryl alcohol polyethylene glycol ether available commercially under the trade name Glycofurol; or (especially) 1,2-propylene glycol; or dimethylisorbide, and may optionally further include lower alkanols, e.g., ethanol.

Suitable components for the lipophilic phase include medium chain fatty acid triglycerides, mixed mono-, di-, and tri-glycerides, and transesterified ethoxylated vegetable oils, especially purified mono-, di-, tri-glycerides from glycerolysed corn oil e.g., free or substantially free from glycerol and saturated fatty acid components, e.g. as described and claimed in GB 2 284 615 B, the contents of which are incorporated herein by reference.

In one embodiment the lipophilic phase comprises a transesterification product of corn oil and glycerol having a saturated fatty acid content of mono-, di-, and tri-glycerides, and having a glycerol content less than 10% by weight, e.g. less than 5% such as 2 % or less. The transesterification product comprises

i) from about 25% to about 50% by weight, e.g. 30% to 40%, of mono-glycerides; from about 30% to about 60%, e.g. about 45% to about 55%, by weight of di-glycerides; and at least

5% by weight of tri-glycerides, e.g. about 7.5 to about 15%;

ii) a linoleic acid, oleic acid and linolenic acid mono-, di- and tri-glyceride content of at least 85% by weight; and

the total saturated fatty acid content of mono-, di-, and tri-glycerides is less than 10% by weight. In a preferred embodiment the transesterification product has a total palmitic acid and stearic acid content of mono-, di- and tri-glycerides of less than 10% by weight.

Suitable surfactants include reaction products of natural or hydrogenated vegetable oils and ethylene glycol, e.g., polyethoxylated castor oils available for example under the trade mark CREMOPHOR (H.P. Fiedler, Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete, Vol. 1, 3rd edition, 1989) e.g. CREMOPHOR RH 40 or EL; polyoxyethylene-glycerol-fatty acid esters, e.g. available under the trade mark TAGAT, e.g. TAGAT TO; and polyoxyethylene sorbitan fatty acid esters, e.g. mono-, di- and tri-lauryl, palmityl, stearyl and oleyl esters of the type known and available under the trade mark TWEEN, e.g. TWEEN 40 or TWEEN 80. These and other surfactants are also described in GB 2 222 770 and GB 2 257 359.

The compositions may optionally further comprise flavoring agents and/or antioxidants e.g. α -tocopherol typically in an amount of from 0.05% to about 5% by weight, preferably from about 0.1 to about 1% by weight based on the weight of the composition. The compositions may comprise acidic stabilizing agents, e.g. malonic acid, oxalic acid, citric acid or lactic acid e.g. in an amount of from 0.05% to about 5% by weight, preferably from about 0.1 to about 1% by weight based on the weight of the composition.

The pharmaceutical compositions are preferably compounded in unit dosage form, for example by filling into orally administrable capsule shells. The capsule shells may be soft or hard gelatine capsule shells. Stable soft gelatin capsules containing for example the cyclosporin A / 40-O-(2-hydroxyethyl)-rapamycin compositions of this invention may be prepared in accordance with the method described in GB 2 282 586, the contents of which are incorporated herein by reference. If desired, however, the pharmaceutical compositions may be in drink solution form and may include water or any other aqueous system, to provide emulsion or microemulsion systems suitable for drinking.

Depending on the carrier material used, a solid dispersion in the form of a simple eutectic mixture, solid solution or solid micellar solution, glass suspension or glass solution of active agent/complex association may be formed which contains the IL-2 transcription inhibitor, e.g.

cyclosporin A, with 40-O-(2-hydroxyethyl)-rapamycin in fine to molecularly dispersed form. Thus in one embodiment, a solid dispersion is formed which is a co-precipitate of the IL-2 transcription inhibitor, e.g. cyclosporin A, with 40-O-(2-hydroxyethyl)-rapamycin and a carrier medium e.g. as described in PCT/EP96/03066 the contents of which are incorporated herein by reference. In the solid dispersion, the IL-2 transcription inhibitor, e.g. cyclosporin A, and 40-O-(2-hydroxyethyl)-rapamycin are in amorphous or substantially amorphous form, and are physically bound to the carrier medium. The carrier medium, typically present in an amount of between 10 and 99.5 % by weight based on the total weight of the composition, may comprise a water-soluble polymer for example hydroxypropylmethylcellulose, hydroxypropylmethylcellulose phthalate, polyvinylpyrrolidone, hydroxypropylcellulose or derivatives thereof; a polyethylene glycol, for example PEG 2000, PEG 4000 or PEG 6000 (Handbook of Pharmaceutical Excipients); a saturated polyglycolised glyceride, available for example under the trade mark Gelucir, e.g. Gelucir 44/14, 53/10, 50/13, 42/12, or 35/10; or a cyclodextrin, for example a β -cyclodextrin or an α -cyclodextrin.

The carrier medium may further comprise a surfactant, for example as described above, or a polyoxyethylene-polyoxypropylene co-polymer or block co-polymer known, for example, under the trade names Pluronic or Poloxamer, e.g. Poloxamer 188; an ethoxylated cholesterol for example Solulan C24; a vitamin derivative, e.g. tocopherol polyethylene glycol succinate; sodium dodecylsulfate or sodium laurylsulfate; a bile acid or salt thereof, for example cholic acid, glycolic acid or a salt, e.g. sodium cholate; or lecithin. If present in the solid dispersion, the surfactant is generally in an amount of up to 20% by weight based on the total weight of the composition, e.g. 1 to 15% by weight. Other pharmaceutically acceptable excipients, e.g. as described above, may be included in the solid dispersion as desired. When formulated as a solid dispersion, the compositions of this invention may be administered, for example, in tablet, capsule, granule or powder form, e.g. in a sachet.

In a further aspect the invention provides a pharmaceutical composition in the form of a microemulsion or a microemulsion preconcentrate, or a solid dispersion, e.g. as described herein, comprising (i) a rapamycin, e.g. rapamycin or 40-O-(2-hydroxyethyl)-rapamycin, and (ii) an IL-2 transcription inhibitor, e.g. cyclosporin A or FK 506, preferably in a synergistic ratio, e.g. as described above, for use in treatment or prevention of a condition as described above, e.g., the

treatment or prevention of an autoimmune or inflammatory condition, or transplant rejection, especially chronic rejection or xenograft rejection, for example,

A pharmaceutical composition having cyclosporin A and rapamycin in a synergistically effective ratio in an oil-in-water microemulsion or in a water-in-oil microemulsion preconcentrate capable of forming a microemulsion, comprising a hydrophilic phase, a lipophilic phase, and a surfactant, e.g. wherein the hydrophilic phase, lipophilic phase, and surfactant are as described in GB 2 222 770, GB 2 257 359 or in WO 96/13273 the contents of which publications are incorporated herein by reference, e.g., wherein the hydrophilic phase may comprise 5 to 50 % by weight of the composition, e.g. 10 to 50%, preferably 15 to 40 % by weight; the lipophilic phase may comprise 5 to 85 % by weight of the composition, e.g. 10 to 85%, preferably 15 to 70 % by weight; and the surfactant may comprise 5 to 80 % by weight of the composition, preferably 10 to 70% by weight; or

A pharmaceutical composition having cyclosporin A and rapamycin in a synergistically effective ratio in the form of a solid dispersion, e.g., a co-precipitate of cyclosporin A with rapamycin and a carrier medium e.g. as described above and in PCT/EP96/03066 the contents of which are incorporated herein by reference, and optionally further comprising a surfactant, for example as described above, e.g., in an amount of up to 20% by weight based on the total weight of the composition, e.g. 1 to 15% by weight.

Microemulsions, microemulsion preconcentrates and solid dispersions comprising rapamycins other than 40-O-(2-hydroxyethyl)-rapamycin, e.g., rapamycin, may be formulated and used as described herein for compositions comprising 40-O-(2-hydroxyethyl)-rapamycin.

EXAMPLE 1 - Synergism between 40-O-(2-hydroxyethyl)-rapamycin and cyclosporin A in vitro

Synergy is shown between the 40-O-(2-hydroxyethyl)-rapamycin and cyclosporin A in the two-way mouse mixed lymphocyte reaction. Spleen cells of BALB/c and CBA mice (1×10^5 from each strain) are cultured in duplicate in flat-bottom 96-well microtiter plates in the absence or presence of serially diluted compound in serum-free medium. After four days, ^3H -thymidine is added. Sixteen hours later, the cells are harvested and ^3H -thymidine incorporation is measured by liquid scintillation counting. Cell proliferation is assessed in the absence of compounds (100% value) or in the presence of serially diluted compounds or combinations of compounds. The maximum ^3H thymidine incorporation (100% value) is around 240×10^3 cpm, and the background

^3H -thymidine incorporation in unstimulated cells is around 5×10^3 cpm. Drug concentrations at which the maximum proliferative response is inhibited by 70% (IC_{70}) are calculated using a four-parameter logistic function. The IC_{70} is 21 nM for cyclosporin A and 0.3 nM for 40-O-(2-hydroxyethyl)-rapamycin. In combinations, lymphocyte proliferation is determined in the presence of 0.05, 0.1, 0.2, 0.4, and 0.8 nM 40-O-(2-hydroxyethyl)-rapamycin, together with 2.5, 5.0, 10, 15, 20, or 25 nM cyclosporin A. For each drug combination, the respective IC_{70} of each individual compound is calculated. These IC_{70} values are converted into relative units with regard to the IC_{70} value of the respective single drug treatment. These relative IC_{70} units are graphically presented in an isobologram in Fig. 1. The concave nature of this isobologram indicates a synergistic action between the two compounds. The index of synergy at the most synergistic combination is calculated at a value of 0.8, e.g., a value of A/A_E of 0.19 for 40-O-(2-hydroxyethyl)-rapamycin and 0.52 for cyclosporin A.

EXAMPLE 2: Synergism between 40-O-(2-hydroxyethyl)-rapamycin and cyclosporin A in vivo

Synergism in vivo is shown using orthotopic kidney and heterotopic heart allotransplantation models in male Lewis rats (RT1^1 haplotype) using donor organs from male DA rats (RT1^k haplotype).

Kidney transplantation is followed by contralateral nephrectomy 7 days later: at nephrectomy, the graft is macroscopically inspected, and if rejection is macroscopically evident, the experiment is terminated. Surviving animals are monitored daily for clinical signs of renal dysfunction.

The heart allografts are transplanted into the abdomen, with anastomoses between the donor aorta and the recipient infrarenal abdominal aorta and between the donor right pulmonary artery and the recipient inferior vena cava. The abdomen is then palpated daily to determine if the graft is still beating; in case of cessation of heartbeat, the experiment is terminated.

In both the kidney and heart allograft experiments, the termination point in long term survivors is 100 days. In all cases, the graft is removed at autopsy, fixed in buffered formalin, and embedded in paraffin. Four micron thick sections stained with hematoxylin and eosin are read for signs of rejection and scored as "no rejection", or "marginal", "slight", "moderate", or "severe" cellular rejection based on the extent of mononuclear cell infiltration and damage to the parenchyma (tubules in the kidney, myocytes in the heart).

Lewis rats left untreated after transplantation reject a kidney allograft within 7 days, with a histology of severe cellular rejection; the heterotopic heart allograft in untreated recipients stops beating between day 7 and 10 post transplantation, with a similar histology of severe cellular rejection.

Cyclosporin A (NEORAL) and/or 40-O-(2-hydroxyethyl)-rapamycin are given daily orally in a microemulsion preconcentrate vehicle. Allograft survival data and histology are presented in Table 1. The minimum effective dose of cyclosporine A giving long term allograft survival is 5.0 mg/kg body weight, with a slight rejection in graft histology. For 40-O-(2-hydroxyethyl)-rapamycin, a dose of 5.0 mg/kg must be given for long term survival of a kidney allograft; this dose is not fully effective for heart allograft.

In combination treatment, a dose of 1.0 or 2.0 mg/kg cyclosporin A is combined with dosages of 0.5, 1.0 or 2.0 mg/kg 40-O-(2-hydroxyethyl)-rapamycin. Long term survival of the kidney allograft is seen at the lowest dosages of both compound; long term survival of the heart allograft at 1.0 mg/kg of each compound. Moreover, the histology of the long term surviving allografts is improved. Based on the minimum effective dose giving long term survival in single compound treatment (5.0 mg/kg for cyclosporin A, > 5.0 mg/kg for 40-O-(2-hydroxyethyl)-rapamycin), the index of synergy for the kidney transplant model is about 0.3 (1.0 mg/kg cyclosporin A) to 0.5 (2.0 mg/kg cyclosporin A), and for heart transplant, <0.5 (1.0 mg/kg cyclosporin A) and <0.7 (2.0 mg/kg cyclosporin A). Thus the in vivo synergy of the compounds is even greater than would be predicted from the in vitro results.

Table 1. Effect of Neoral ® and 40-0-(2-hydroxyethyl)-rapamycin				
40-0-(2-hydroxyethyl) rapamycin dose (mg/kg)	Neoral ® dose (mg/kg)	n	Survival (days)	Histology of rejection
KIDNEY				
	2.5 ^a	3	7*,7*,7*	severe
	5.0 ^a	2	24≥100	24:severe 100:slight
	7.5 ^a	6	≥90 (3x), ≥100 (3x)	marginal, slight
1.0		3	11,13,18	moderate-severe
2.5		4	14,15,18,18	slight-moderate
5.0		3	42, ≥100, ≥100	moderate; ≥100 marginal
0.5	1.0	3	≥100, ≥100, ≥100	marginal-slight
1.0	1.0	6	12,17, ≥100 (4x)	marginal-slight
2.0	1.0	3	≥64, ≥100, ≥100.	marginal
0.5	2.0	3	≥100, ≥100, ≥100	no rejection
1.0	2.0	3	≥72, ≥84, ≥100	no rejection
2.0	2.0	3	≥73, ≥100, ≥100	no rejection
HEART				
	2.5	3	10,10,14	severe
	5.0	3	≥100, ≥100, ≥100	slight
1.0		3	12,14,14	severe
2.5		6	16,18,22,25,27, ≥28	moderate
5.0		2	22,23	moderate
0.5	1.0	3	18,18,19	moderate
1.0	1.0	5	18, ≥93, ≥93, ≥95, ≥105	slight-moderate
2.0	1.0	3	≥91, ≥91, ≥92	marginal
1.0	2.0	3	≥78, ≥92, ≥106	no rejection
2.0	2.0	3	≥66, ≥105, ≥106	no rejection

^a 14-day daily treatment

EXAMPLE 3 - Pharmacokinetic interaction

³H-labeled 40-O-(2-hydroxyethyl)-rapamycin is given to male rats together with ¹⁴C-labeled Cyclosporin A, either as an intravenous bolus (1 mg/kg and 3 mg/kg, respectively) or orally in microemulsion (1.5 mg/kg and 3 mg/kg, respectively). Whole blood concentrations of both compounds are determined by liquid chromatography - reversed isotope dilution (LC-RID). The interaction between 40-O-(2-hydroxyethyl)-rapamycin and Cyclosporin A is investigated by comparing the pharmacokinetics after coadministration with those obtained after administration of each test compound alone, with results as depicted in Tables 2 and 3.

TABLE 2

40-O-(2-hydroxyethyl)-rapamycin and Cyclosporin disposition after intravenous administration alone and in combination

Doses: 1 mg/kg [^3H]40-O-(2-hydroxyethyl)-rapamycin, 3 mg/kg [^{14}C] Cyclosporin (values are means \pm SE)

Parameter		40-O-(2-hydroxyethyl)-rapamycin alone	40-O-(2-hydroxyethyl) rapamycin (with Cyclosporin)	Cyclosporin alone	Cyclosporin (with 40-O-(2-hydroxyethyl)-rapamycin)
AUC	$\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{h}$	0.82 ± 0.04	1.55 ± 0.16	12.5 - 19.0	11.9 ± 1.5
$t_{1/2}$	h	60 ± 10	56 ± 7	16 - 19	24 ± 1
CL	ml/min	8.2 ± 0.3	3.2 ± 0.3	0.7 - 1.4	1.3 ± 0.1
V	l/kg	52 ± 3	25 ± 3	2.7 - 4.5	3.3 ± 0.3

TABLE 3

Pharmacokinetics after oral administration alone and in combination

Doses: 1.5 mg/kg [^3H], 3mg/kg [^{14}C]Cyclosporin (values are means \pm SE)

Parameter		40-O-(2-hydroxyethyl)-rapamycin alone	40-O-(2-hydroxyethyl)-rapamycin (with Cyclosporin)	Cyclosporin alone	Cyclosporin with 40-O-(2-hydroxyethyl) rapamycin
C_{max}	ng/ml	16.7 ± 4.6	27.4 ± 7.3	228 ± 111	438 ± 247
t_{max}	h	1.6 ± 0.7	0.8 ± 0.2	0.5 ± 0	3.5 ± 2.3
$t_{1/2}$	h	61 ± 5	30 ± 6	12 ± 1	17 ± 3
AUC	$\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{h}$	0.18 ± 0.06	0.31 ± 0.08	1.00 ± 0.20	3.10 ± 1.65

Clearance and volume of distribution of 40-O-(2-hydroxyethyl)-rapamycin are decreased (two-fold) by coadministration with Cyclosporin A; on the other hand, the disposition of Cyclosporin A is not affected by 40-O-(2-hydroxyethyl)-rapamycin. The increase of blood levels of 40-O-(2-hydroxyethyl)-rapamycin (two-fold) observed after oral coadministration with Cyclosporin may be attributed to the decrease in clearance and volume of distribution, whereas the two-fold increase of Cyclosporin blood levels after oral coadministration with 40-O-(2-hydroxyethyl)-rapamycin may be attributed to a higher absorption.

EXAMPLE 4 - Combined formulation

A soft gelatin capsule suitable for oral administration is prepared containing the following composition in a 500mg dosage

Cyclosporin A	50 mg
40-O-(2-hydroxyethyl)-rapamycin	5 mg
1,2-propylene glycol	50 mg
ethanol absolute	50 mg
corn oil mono-,di-,tri-glycerides	154.5 mg
Cremophor RH40	190 mg
DL-alpha-tocopherol	<u>0.5 mg</u>
Total	<u>500 mg</u>

The composition is stable and no precipitation is observed.

Examples 5 to 9 - combined formulations

Soft gelatin capsules suitable for oral administration are prepared containing the following compositions, each in a 500mg dosage

Example	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>
(amounts are in mg)					
Cyclosporin A	48	49	51	52	53
40-O-(2-hydroxyethyl)-rapamycin	7	6	4	3	2
1,2-propylene glycol	50	50	50	50	50
ethanol absolute	50	50	50	50	50
corn oil mono-,di-,tri-glycerides	154.5	154.5	154.5	154.5	154.5
Cremophor RH40	190	190	190	190	190
DL-alpha-tocopherol	0.5	0.5	0.5	0.5	0.5

The compositions are stable and no precipitation is observed.

Example 10

A solid dispersion formulation is prepared containing the following components in parts by weight:

Cyclosporin A	10
40-O-(2-hydroxyethyl)-rapamycin	1
HPMC 3 cps	75
Poloxamer 188	14

The components are dissolved in a 1:1 absolute ethanol/acetone mixture. The solvents are evaporated and the resulting dry residue milled to a fine powder with mean particle size around 0.1 to 0.4 mm. The fine powder is tableted or filled into hard capsules.

CLAIMS

1. A method of treatment or prevention of an autoimmune or inflammatory condition or transplant rejection in a subject suffering from or at risk for such condition or rejection, comprising co-administering synergistically effective amounts of (i) an IL-2 transcription inhibitor and (ii) 40-O-(2-hydroxyethyl)-rapamycin.
2. The method according to claim 1 wherein the IL-2 transcription inhibitor is cyclosporin A.
3. The use of an IL-2 transcription inhibitor in the manufacture of a medicament for co-administration in synergistically effective amounts with 40-O-(2-hydroxyethyl)-rapamycin.
4. The use of 40-O-(2-hydroxyethyl)-rapamycin in the manufacture of a medicament for co-administration in synergistically effective amounts with an IL-2 transcription inhibitor.
5. The use according to claim 3 or 4 wherein the IL-2 transcription inhibitor is cyclosporin A.
6. A kit of parts comprising an IL-2 transcription inhibitor and 40-O-(2-hydroxyethyl)-rapamycin in separate unit dosage forms, wherein said unit dosage forms are suitable for administration of the two compounds in synergistically effective amounts, together with instructions for use.
7. The kit according to claim 6 wherein the IL-2 transcription inhibitor is cyclosporin A.
8. The kit as claimed in claim 6 or claim 7 further comprising means for facilitating compliance with the administration.
9. The use of an IL-2 transcription inhibitor in the manufacture of a pharmaceutical kit which is to be used for facilitating co-administration with 40-O-(2-hydroxyethyl)-rapamycin.

10. The use of 40-O-(2-hydroxyethyl)-rapamycin in the manufacture of a pharmaceutical kit which is to be used for facilitating co-administration with an IL-2 transcription inhibitor.
11. The use as claimed in claim 9 or claim 10 wherein the IL-2 transcription inhibitor is cyclosporin A.
12. An IL-2 transcription inhibitor and 40-O-(2-hydroxyethyl)-rapamycin as a combined pharmaceutical preparation for simultaneous, separate or sequential use in synergistically effective amounts.
13. The pharmaceutical preparation according to claim 12 wherein the IL-2 transcription inhibitor is cyclosporin A.
14. A pharmaceutical composition comprising an IL-2 transcription inhibitor and 40-O-(2-hydroxyethyl)-rapamycin in combination or association with a pharmaceutically acceptable diluent of carrier.
15. A pharmaceutical composition in the form of a microemulsion or a microemulsion concentrate or a solid dispersion, comprising an IL-2 transcription inhibitor and a rapamycin.
16. A pharmaceutical composition according to claim 14 or 15 which is a microemulsion concentrate formulation.
17. A pharmaceutical composition according to claim 14 or 15 which is a solid dispersion formulation.
18. A pharmaceutical composition according to any one of claims 14 to 17 wherein the IL-2 transcription inhibitor is cyclosporin A.
19. A pharmaceutical composition as claimed in claim 15 or 17 wherein the rapamycin is 40-O-(2-hydroxyethyl)-rapamycin.

20. A pharmaceutical composition according to any one of claims 14 to 16 wherein cyclosporin A and 40-O-(2-hydroxyethyl)-rapamycin are present in a synergistically effective ratio in an oil-in-water microemulsion preconcentrate comprising a hydrophilic phase, a lipophilic phase, and a surfactant.

21. All novel products, processes and utilities substantially as hereinbefore described, particularly with reference to the examples.

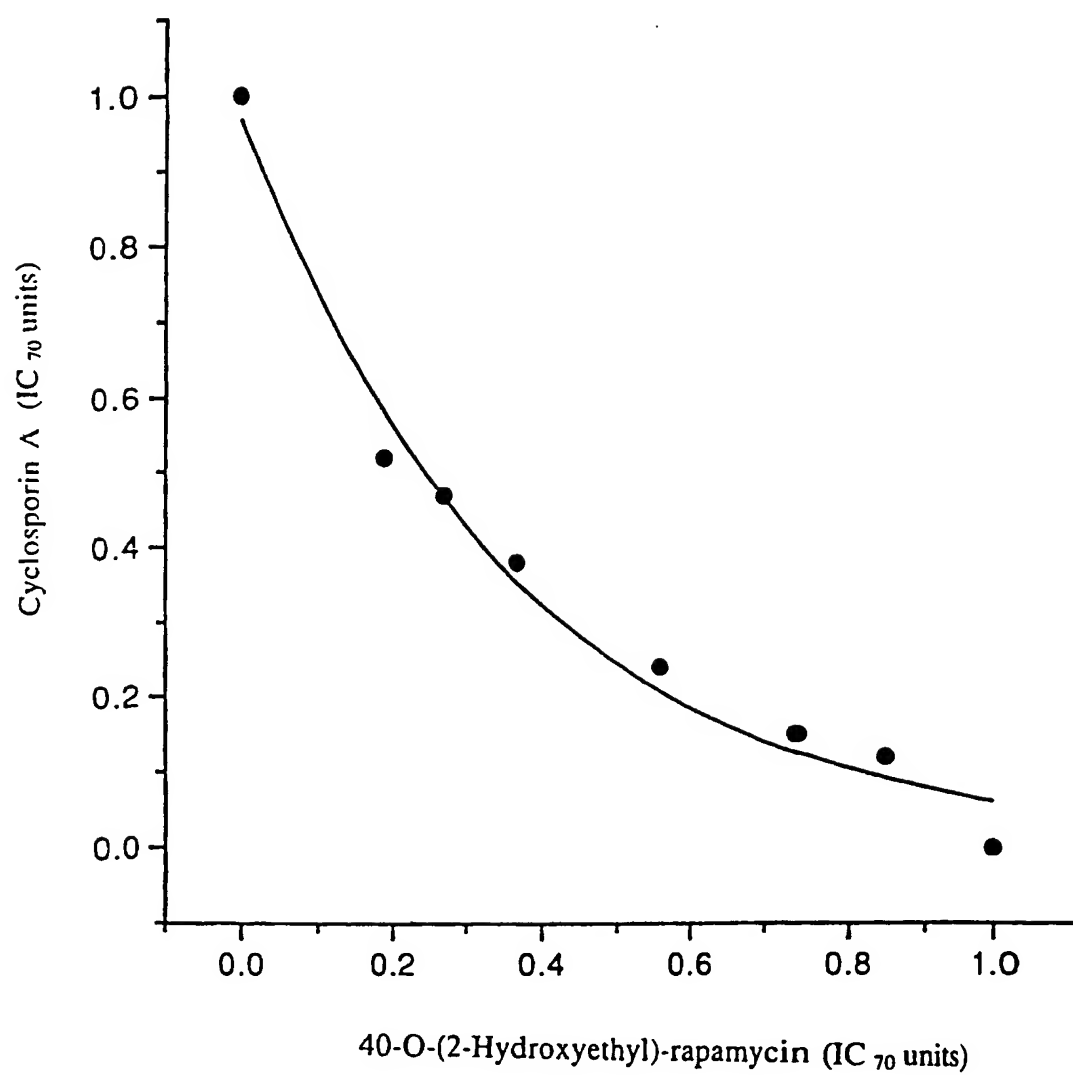


Figure 1

INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/EP 97/04123

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K38/13 A61K31/445 A61K31/71 //(A61K38/13,31:71), (A61K38/13,31:445), (A61K31/71,31:71), (A61K31/71,31:445), (A61K31/445,31:445)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SHUURMAN H.J. ET AL: "SDZ-RAD, A NEW RAPAMYCIN DERIVATIVE. SYNERGISM WITH CYCLOSPORINE" TRANSPLANTATION, vol. 64, no. 1, 15 July 1997, pages 32-35, XP002048030 see abstract see page 34, column 1, paragraph 2 - column 2, paragraph 2; table 1 ---	1-5,9-20
Y	TU Y. ET AL: "The synergistic effects of cyclosporine, sirolimus, and brequinar on heart allograft survival in mice" TRANSPLANTATION, 1995, 59/2 (177-183), USA, XP002048031 see abstract; figure 2; tables 3,4 see page 177, column 2, paragraph 4 --- -/--	1-20
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
<div style="display: flex;"> <div style="flex: 1;"> <p style="font-size: small;">* Special categories of cited documents:</p> <p style="font-size: x-small;">"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p style="font-size: x-small;">"E" earlier document but published on or after the international filing date</p> <p style="font-size: x-small;">"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p style="font-size: x-small;">"O" document referring to an oral disclosure, use, exhibition or other means</p> <p style="font-size: x-small;">"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p style="font-size: x-small;">"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p style="font-size: x-small;">"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p style="font-size: x-small;">"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p style="font-size: x-small;">"A" document member of the same patent family</p> </div> </div>		
2	Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">25 November 1997</div>	Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">09.01.98</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-weight: bold;">Gonzalez Ramon, N</div>

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/04123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	SCHULER W. ET AL: "SDZ-RAD, A NEW RAPAMYCIN DERIVATIVE. Pharmacological properties in vitro and in vivo" TRANSPLANTATION, vol. 64, no. 1, 15 July 1997, pages 36-42, XP002048032 see abstract see page 40, column 1 - page 41, column 1 ---	1-5, 12-14
P,Y	WO 97 25977 A (CIBA GEIGY AG ;TIEMESSEN HARRY (DE)) 24 July 1997 see abstract see page 3, paragraph 2-4 see page 13, paragraph 5 - page 14, paragraph 3 ---	6-11, 15-20
A	WO 94 09010 A (SANDOZ AG ;SANDOZ AG (DE); SANDOZ LTD (CH); COTTENS SYLVAIN (CH);) 28 April 1994 cited in the application see abstract see page 5, paragraph 4 - page 7, paragraph 1; claims 1,2,7,8; example 8 ---	1-20
A	EP 0 401 747 A (CALNE ROY) 12 December 1990 see abstract see page 4, line 20-50; claims 1-4 -----	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/04123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 21

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Obscure definition of products, processes and utilities as 'substantially as herein before described'

Remark : Although claims 1,2 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat'l Application No

PCT/EP 97/04123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9725977 A	24-07-97	AU 1543497 A	11-08-97
WO 9409010 A	28-04-94	AU 676198 B	06-03-97
		AU 4819293 A	09-05-94
		CA 2145383 A	28-04-94
		CZ 9500899 A	13-09-95
		EP 0663916 A	26-07-95
		FI 951678 A	07-04-95
		HU 71232 A	28-11-95
		JP 8502266 T	12-03-96
		NO 951312 A	08-06-95
		NZ 256026 A	27-08-96
		PL 308268 A	24-07-95
		SK 46595 A	09-08-95
		US 5665772 A	09-09-97
EP 0401747 A	12-12-90	US 5100899 A	31-03-92
		AT 135215 T	15-03-96
		AU 638253 B	24-06-93
		AU 5686590 A	31-01-91
		CA 2018287 A	06-12-90
		DE 69025803 D	18-04-96
		DE 69025803 T	25-07-96
		ES 2085299 T	01-06-96
		IL 94634 A	31-03-96
		JP 3073158 A	28-03-91
		MX 173183 B	07-02-94
		US 5308847 A	03-05-94
		US 5403833 A	04-04-95
		US 5461058 A	24-10-95
		US 5212155 A	18-05-93